Alpha-1 Antitrypsin Regulates Transcriptional Levels of Serine Proteases in Blood Mononuclear Cells

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Alpha1-antitrypsin regulates transcriptional levels of serine proteases in blood mononuclear cells

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To the Editor:

PiZZ (Glu342Lys) alpha1-antitrypsin deficiency (A1ATD) is a typical genetic risk factor associated with the development of early onset COPD with emphysema. Because A1AT is a major circulating inhibitor of serine proteases (serpin), a severe deficiency of this protein may lead to lung tissue damage by uncontrolled activity of neutrophil elastase, proteinase 3 and other serine proteases. In general, a proteinase/antiproteinase imbalance is among potential mechanisms implicated in the pathophysiology of COPD (1). The importance of A1AT in maintaining protease-antiprotease homeostasis is also supported by a positive correlation between a recently described *in vivo* marker of neutrophil elastase activity (Aα-Val360) and disease severity in emphysema related to A1ATD (2). There is however a considerable heterogeneity in the clinical expression among people with type ZZ A1ATD. Some develop emphysema in early adulthood (35-45 years of age) while others in late adulthood or not at all, and severity of symptoms also varies.

Peripheral blood mononuclear cells (PBMCs) have emerged in recent years as surrogate markers of several diseases, including preeclampsia, rheumatoid arthritis and malignant diseases (3, 4). COPD is also characterized by the altered features of PBMC. The dysfunction of PBMCs has been linked to acute exacerbations in COPD (5). We previously found that blood monocytes of COPD patients release more MMP-9 and IL-6, and showed NF-κB activation compared to healthy controls (6). Current studies suggest that gene expression signatures in PBMCs could serve as markers of disease activity or expression in COPD (7).

We hypothesized that the PBMCs may have specific gene expression signatures that are related to clinically healthy PiZZ not present in PBMCs of PiMM (normal A1AT gene) carriers. To address this, we isolated cells from 8 PiZZ asymptomatic donors matched with 12 PiMM healthy donors (see Table 1). Lung function tests and routine clinical laboratory analyses, including determination of serum A1AT concentration and genotype were performed at the Department of Internal Medicine, Philipps-Universität Marburg, Germany. Every donor gave written informed consent for collection and use of blood samples for this study. The study was approved by the Marburg University ethics committee.

The PBMC were isolated using lymphosep discontinuous gradient centrifugation, resuspended in RPMI-1640 with 2 mM N-acetyl-L-alanyl-L-glutamine (Gibco, Life Technologies) and incubated for 75 min at 37°C and 5% CO_2 to allow monocytes to adhere to the cell culture plates. Afterwards, non-adherent cells were removed, adherent PBMCs were

used for the mRNA preparation. Gene expression analysis by reverse-transcription quantitative PCR (RT-qPCR) was assessed as described earlier using two internal housekeeping genes, Glucuronidase β (GUSB) and β-actin (ACTB) (8). Statistical Package (SPSS for Windows, release 21.0) was used for the statistical calculations.

Our results indicate that adherent PiZZ PBMCs from asymptomatic donors express significantly higher levels of elastase (ELANE), proteinase 3 (PR3) and cathepsin G if compared to PBMCs from healthy PiMM donors (see Table 1). The relative expression of A1AT (SERPINA1) was slightly (by about 38%) lower in PiZZ than in PiMM PBMCs ($p<0.05$). Moreover, relative expression of SERPINA1 gene inversely correlates with expression of ELANE ($r=-0.82$, $p=0.001$), PR3 ($r=-0.82$, $p=0.001$) and cathepsin G ($r=-0.72$, $p=0.006$) in PBMCs. This latter finding implies that A1AT probably regulates the expression of serine proteases. To provide an additional support for this concept, we prepared mRNA from adherent PBMCs of 10 PiZZ COPD patients who were on long-term infusion of plasma purified A1AT protein (Prolastin®, Grifols, Spain). Blood for PBMCs isolation was taken just before patients received their next weekly infusion. Quantitative real-time PCR analysis was employed to assess the expression of ELANE, PR3, cathepsin G and SERPINA1. As shown in **Table 1**, PiZZ PBMCs from patients treated with Prolastin® showed lower expression of all three enzymes relative to PiZZ PBMCs from healthy donors. Specifically, ELANE expression was lower by 50%. As expected, serum levels of A1AT were as following PiMM > PiZZ with Prolastin®> PiZZ no Prolastin® and were inversely related to the expression levels of ELANE ($r=-0.58$, $p=0.004$), PR3 ($r=-0.52$, $p=0.014$) and cathepsin G ($r=-0.56$, $p=0.007$). These data further support the notion that A1AT not only inhibits activity but also regulates transcriptional levels of serine proteases. We also sought supportive evidence for our hypothesis by analyzing the ELANE mRNA levels in PBMCs isolated from 12 randomly selected PiZZ COPD patients pre- and post- Prolastin® therapy. Pre-therapy ELANE expression was highly variable and probably concordant with PiZZ COPD heterogeneity. Therefore, we identified two subgroups of patients with expression levels of ELANE below and above one. Significant effect of therapy was only observed in the PBMCs from a subgroup with higher ELANE mRNA [mean (SE):pre-2.49 (0.85) vs post-0.64 (0.25) ($n=5$), Prolastin® $p=0.003$ and pre-0.16 (0.69) vs post-0.11 (0.04), Prolastin® ($n=8$) n.s., respectively]. In this data set, we are able to show a significant benefit in reducing ELANE expression by the A1AT drug. These data need to be verified in larger cohorts, to study if the best effect of A1AT therapy occurs in PiZZ patients with high transcriptional levels of elastase.

Earlier studies have found that elastase upregulates A1AT synthesis, specifically in the mononuclear phagocyte lineage. Perlmutter and coauthors demonstrated that elastase increases levels of type MM A1AT mRNA in monocytes and macrophages, resulting into a concomitant increase in intracellular accumulation of newly synthesized A1AT protein (9). In subjects with PiZZ A1AT, elastase also stimulates monocyte synthesis of Z-A1AT but has no effect on the rate of secretion. Evidently, elastase increases the accumulation of Z-A1AT (9), which leads to monocyte activation (10). Therefore, use of A1AT therapy might provide a double benefit for PiZZ A1AT subjects by reducing serine protease activity and expression. Consequently, A1AT therapy may also indirectly reduce the expression of Z-A1AT (Figure 1). In fact, the transcription of SERPINA1 gene was somewhat lower in PiZZ PBMCs from COPD patients treated with A1AT (Prolastin®) if compared to PiMM or PiZZ PBMCs isolated from healthy donors (see Table 1).

In conclusion, our findings highlight novel benefits of A1AT augmentation therapy in ameliorating protease imbalance in A1ATD. Studies in PiZZ COPD patients with and without A1AT therapy are ongoing to apply our findings as a co-primary outcome to monitor patient`s response to augmentation therapy.

Table 1. Patient characteristics and analysed genes.

Variables	**PiMM n=12**	**PiZZ n=8**	**P value (*)**	**PiZZ emphysema treated with A1AT (Prolastin) n=10**	**P value (**)**
Patient characterstics					
Gender. M/F	6/6	4/4		5/5	
Age. years	54 (11.5)	52 (13.6)	0.74	65.6 (11.7)	0.03
FEV_1 % predicted	105 (11.6)	92.1 (38.2)	0.29	48.1 (16.3)	0.001
AST (U/l)	17.4 (4.8)	22.9 (7.3)	0.06	20.8 (10.8)	0.58
ALT (U/l)	24.5 (8.5)	26.3 (15.7)	0.74	29.7 (10.7)	0.83
ALP (U/l)	58.3 (17.7)	61.5 (20.7)	0.72	63.0 (15.0)	0.96
Glucose (mg/dl)	96.5 (13.6)	93.4 (11.3)	0.60	107.7 (36.5)	0.33
Cholesterol (mg/dl)	248.6 (58.1)	252.3 (46.8)	0.90	200.8 (59.3)	0.70
HDL (mg/dl)	70.0 (22.0)	76.2 (20.7)	0.61	60. 0 (14.4)	0.78
LDL (mg/dl)	147.7 (40.8)	151.5 (41.1)	0.87	129.6 (51.2)	0.19
TG (mg/dl)	156.9 (76.2)	103.2 (52.2)	0.17	141.4 (57.9)	0.11
Ferritin (µg/l)	69.7 (64.7)	67.1 (28.1)	0.92	165 (153.9)	0.001
A1AT (g/l)	1.4 (0.3)	0.4 (0.2)	0.001	0.8 (0.1)	0.012
mRNA expression					
Elastase	4.6 (3.53)	25.6 (20.0)	0.016	11.1 (7.8)	0.04
Proteinase3	9.7 (10.5)	28.0 (15.4)	0.045	17.0 (18)	0.12
Cathepsin G	2.1 (2.2)	8.0 (4.4)	0.030	3.99 (4.9)	0.26
Serpina1	1.3 (0.5)	0.95 (0.5)	0.05	0.84 (0.5)	0.06

Values are Mean (± SD).

* Significance of PiZZ in comparison to PiMM

** Significance of PiZZ emphysema treated with A1AT in comparison to PiZZ healthy

SD. Standard deviation; M. male; F. female; $FEV_{1.}$ forced expiratory volume in 1sec; AST. aspartate aminotransferase; ALT. alanine aminotransferase; ALP. alkaline phosphatase; HDL. high density lipoprotein; LDL. low density lipoprotein; TG. triglycerides; A1AT. alpha1-antitrypsin

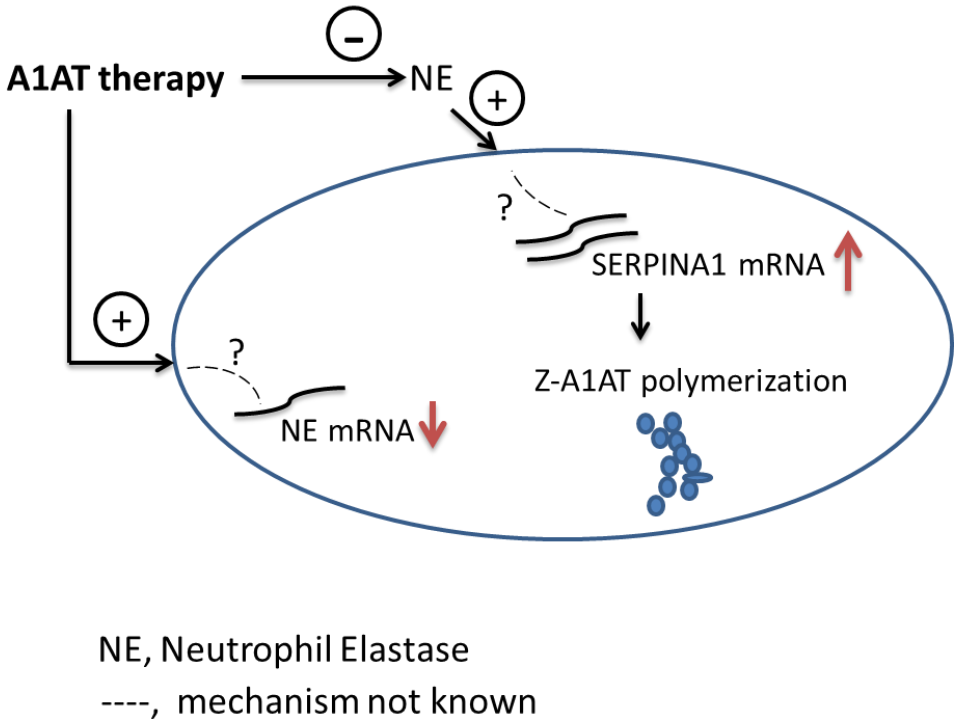

Figure 1. Effect of A1AT therapy on elastase and Z-A1AT expression in monocytes/alveolar macrophages (schematic presentation of our hypothesis).

Our results show that adherent PBMCs isolated from healthy persons carrying PiZZ A1ATD express significantly higher levels of elastase than PiMM PBMCs. NE has been found to induce synthesis of Z-A1AT in PiZZ monocytes without affecting the rate of protein secretion; hence, the enzyme increases the intracellular accumulation of pathogenic polymers of Z-A1AT. The proteolytic function of NE is blocked by A1AT through covalent binding to each other; thus, A1AT protects tissues from serine protease-induced damage. However, in A1ATD subjects uncontrolled elastase activity due to the low levels of functional Z-A1AT might enhance Z-A1AT synthesis leading to the accumulation of intracellular pathogenic Z-A1AT polymers and persistant innate immune cells activation. Under chronic inflammatory conditions a vicious circle might be generated. Therapy with plasma purified A1AT will normalize levels of A1AT to inhibit NE activity but also reduce NE transcriptional levels. A1AT therapy may also indirectly reduce the expression of Z-A1AT.

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